Environmental Health Perspectives Supplements
Vol. 101 (Suppl. 3): 109–112 (1993)

Polymorphism in *CYP1A1* and *CYP2D6* Genes: Possible Association with Susceptibility to Lung Cancer

by Ari Hirvonen,[1] Kirsti Husgafvel-Pursiainen,[1] Sisko Anttila,[1] Antti Karjalainen,[1] and Harri Vainio[1,2]

Polymorphism of *CYP2D6* gene encoding for debrisoquine hydroxylase was determined genotypically for 94 controls and 77 lung cancer patients using a polymerase chain reaction-based application. Both of the point mutations that give rise to deficient alleles of the *CYP2D6* gene are detectable by this method. Out of the 94 healthy controls, 3 individuals (3.2%) had poor metabolizer (PM) genotypes, whereas no PM genotypes were detected in the lung cancer patient group. We observed no difference in the allelic frequencies for either homozygous extensive metabolizers (EMs) or heterozygous EMs between the lung cancer patients and the healthy controls. However, the absence of the poor metabolizer genotype (0/77) in the lung cancer patients is compatible with the hypothesis that there is an increased risk of lung cancer for individuals who are extensive metabolizers of debrisoquine. Another member of the cytochrome P450 gene superfamily that has attracted interest for its potential role in human pulmonary carcinogenesis is the *CYP1A1* gene. In *CYP1A1* gene studies, a polymorphic site assessable to *Msp*I gives rise to two different hybridizable fragments in a Southern blot analysis (alleles *C1* and *C2*, respectively). The *C2C2* genotype has previously been associated with an increased risk of lung cancer. So far 74 lung cancer patients, 30 patients with lung diseases other than cancer, and 118 healthy controls have been studied for *CYP1A1* gene polymorphism. No association between the *Msp*I restriction fragment length polymorphism in the *CYP1A1* gene and lung cancer susceptibility has been found.

Introduction

Individual differences in the ability to metabolize xenobiotics may be a key factor in the genetic predisposition, or host susceptibility, to various carcinogens (*1,2*). The cytochrome P450-dependent mono-oxygenases are important in the metabolism of environmental carcinogens. Among the most studied genetic variables concerning P450 enzymes are the polymorphisms of the genes *CYP2D6* and *CYP1A1*, which encode for debrisoquine 4-hydroxylase and aryl hydrocarbon hydroxylase, respectively (*3*). Both polymorphisms have recently been associated with susceptibility to lung cancer (*1,2*).

The debrisoquine metabolism phenotype has been determined conventionally by administering the test drug and subsequently determining the urinary metabolic ratio (*4*). However, this phenotyping procedure has some limitations because of confounding and the potential adverse effects of the drugs (*5*). Extensive metabolizers (EMs) can either be homozygous or heterozygous for the normal allele, the metabolic ratio being lower in the latter, but the two genotypes cannot be reliably distinguished by phenotyping (*6*).

Recently, a functional *CYP2D6* allele and two related genes, designated *CYP2D7* and *CYP2D8P* (pseudogene), have been isolated and completely sequenced (*7*). This has allowed *CYP2D6* genotyping using polymerase chain reaction (PCR)-based methods (*8,9*). These methods do not have the same limitations as the conventional phenotyping analyses and they can be accomplished using only tiny amounts of white blood cell DNA.

Another P450 enzyme that is of considerable interest for its potential role in human pulmonary carcinogenesis is aryl hydrocarbon hydroxylase (AHH). AHH activity can be induced by polycyclic aromatic hydrocarbon (PAH) components of cigarette smoke condensate (*10,11*). A significant correlation between high AHH-inducibility and enhanced risk of bronchogenic carcinoma in cigarette smokers was originally reported by Kellerman et al. (*12*), and subsequent studies have led to the conclusion that the cigarette smokers with the high inducibility phenotype are several times more prone to bronchogenic carcinoma than smokers with the low inducibility phenotype (*13*). The

[1]Department of Industiral Hygiene and Toxicology, Institute of Occupational Health, Topeliuksenkatu 41 aA, SF-00250 Helsinki, Finland.
[2]International Agency for Research on Cancer, 150 cours Albert-Thomas, Cedex, F-69372 Lyon, France.
Address reprint requests to A. Hirvonen, Institute of Occupational Health, Topeliuksenkatu 41 aA, SF-00250 Helsinki, Finland.

CYP1A1 gene encoding for AHH thus attracts particular interest for its role in individual inducibility status.

Restriction fragment length polymorphisms (RFLPs) are now commonly used for diagnosing and predicting a growing number of clinical disorders and for human family studies. Determination of the cDNA sequence of the *CYP1A1* gene (*14*) has made it possible to look for the RFLPs in the *CYP1A1* gene too. In the early attempts, no association between RFLP patterns and AHH inducibility was noted (*14*). The recent discovery of *Msp*I polymorphism in the last intron of the *CYP1A1* gene (15), however, has led to the detection of cosegregation of the P450IA1 phenotype and *Msp*I RFLP patterns (*16*). In addition, a strong correlation between enhanced lung cancer risk and *Msp*I RFLP patterns of the *CYP1A1* gene was discovered in a Japanese study (*17*).

In this study, a combined application (*18*) of two recently described PCR-based methods (*8,9*) for determinating debrisoquine hydroxylase polymorphism in leukocyte DNA was used to study the distribution of extensive (EM) and poor metabolizer (PM) genotypes in Finnish lung cancer patients and in control subjects. The method used was estimated to detect at least 95% of the known allelic variants of the PM genotype (*18*). The study populations were also screened for the *Msp*I RFLP in the *CYP1A1* gene.

Subjects and Methods

Subjects

Three groups were examined in this study: the first group comprised 91 healthy blood donors at the Finnish Red Cross Blood Transfusion Service (all males) and 27 volunteers (12 males, 15 females) employed at the Institute of Occupational Health in Helsinki, The other two groups comprised 77 lung cancer patients (64 males, 13 females) and 30 patients with lung diseases other than cancer (21 males, 9 females), respectively.

The patients came to Helsinki University Central Hospital over a 3-year period for surgical pulmectomy or lobectomy because they had suspected, operable lung carcinoma. The mean age for the lung cancer patients was 62 years (SD 9.7), and most of them were either current cigarette smokers or ex-smokers for more than 3 years before surgery. According to interview data, only seven patients had never smoked. The blood samples were obtained the day before surgery. None of the patients had received chemotherapy or radiotherapy. The study design was approved by the local ethics committees.

DNA Analyses

DNAs were isolated from bood samples (10–20 mL) obtained from all the subjects. The DNA samples were analyzed by a PCR-genotyping assay and by a *Msp*I RFLP analysis.

The PCR-based *CYP2D6* genotyping analyses were performed as described elsewhere (*18*). Briefly, genomic DNA was first amplified by PCR using two different oligonucleotide pairs. One of the PCR fragments was then digested with the restriction enzyme *Bst*NI. Absence of the restriction site indicated a specific point mutation. The other fragment was used as a template for two allele specific PCR reactions: one with a wild-type specific primer pair and the other with a mutation specific primer pair, which detected the second point mutation studied. The final samples were separated electrophoretically on a 1.8% agarose gel and visualized by UV-irradiation using ethidium bromide staining.

The *CYP1A1* genotyping analysis on lymphocyte DNA was performed in principle as described by Kawajiri and co-workers (*17*) and given in detail elsewhere (*19*).

Statistical Analyses

The chi-square (contingency tables) test with Yates' correlation was used to test the associations between the different genotypes and cancer incidence, as well as other clinical parameters.

Results and Discussion

The electrophoresis results of the combined PCR-genotyping of the *CYP2D6* gene and the RFLP analysis of the *CYP1A1* gene are shown in Figure 1.

The distribution of different *CYP2D6* genotypes among the lung cancer patients and voluntary blood donor controls is shown in Table 1. All the lung cancer patients studied were assessed as either homozygous for two normal alleles or heterozygous for one normal and one mutant allele (i.e., they all had the EM genotype). Except for the lack of lung cancer patients with a PM genotypes, the allelic frequencies in the two populations were quite similar. The percentage of observed PMs in the control population was 3.2%, which fell well within the range obtained previously in Finnish phenotypic studies (*20*). A PCR-based *CYP2D6* genotyping approach similar to ours was applied in a recent study by Daly and co-workers and yielded a good correlation between the debrisoquine metabolic ratio and genotype (*21*).

The RFLP polymorphism in the *CYP1A1* gene was detected by the presence or absence of the *Msp*I site located in the 3′ end of the gene (*17*) resulting in *C2* and *C1* alleles, respectively. The distribution of the different *CYP1A1* genotypes in the three populations is shown in Table 1. The results indicate no difference in the genotypic frequencies between lung cancer and control populations. Furthermore, neither of the studied genotypes revealed any differences between the various histological subtypes of lung cancer (*18,19*).

These conclusions on the *CYP1A1* genotypes are in contrast to the results of Kawajiri et al. (*17*) who showed a statistically significant association between the rare *C2* allele and certain types of lung cancer. In the Japanese study, frequencies of genotypes among lung cancer patients were significantly different from those among healthy controls. In relation to histological types of lung cancer, the squamous cell carcinoma gave the most

Table 1. Distribution of *CYP2D6* and *CYP1A1* genotypes.

CYP2D6	EM	HEM	PM
Lung cancer patients ($n=77$)	62	15	0
Squamous cell carcinoma ($n=39$)	30	9	0
Adenocarcinoma ($n=30$)	24	6	0
Small cell carcinoma ($n=5$)	5	0	0
Large cell carcinoma ($n=3$)	3	0	0
Healthy controls ($n=94$)	73	18	3
CYP1A1	*C1C1*	*C1C2*	*C2C2*
Lung cancer patients ($n=74$)	62	12	0
Squamous cell carcinoma ($n=38$)	31	7	0
Adenocarcinoma ($n=28$)	23	5	0
Small cell carcinoma ($n=5$)	5	0	0
Large cell carcinoma ($n=3$)	3	0	0
Patients with other lung diseases ($n=30$)	25	4	1
Healthy controls ($n=118$)	91	25	2

Abbreviations: EM, extensive-metabolizer genotype; HEM, heterozygous extensive-metabolizer genotypes; PM, poor-metabolizer genotypes.

remarkable deviation from the genotype frequencies in the healthy controls; about 5-fold risk of *C2C2* type was calculated for this smoking related type of lung tumor. Recently, the Japanese group also reported that the smokers with the susceptible genotype were at a remarkably high risk at a low dose level of cigarette smoking and that the difference between genotypes was reduced at high dose levels (*22*).

In contrast to to the Japanese findings, in a similar study concerning Norwegian lung cancer patients, no statistically significant differences in allelic frequencies or distribution of the *CYP1A1* genotypes were seen (*23*). Furthermore, the proportion of the rare genotype *C2C2* was very small compared to the Japanese population.

In the present preliminary study, which included relatively small study populations, the association of *CYP1A1* and *CYP2D6* genes with individual susceptibility to lung cancer could neither be confirmed nor contradicted. It is

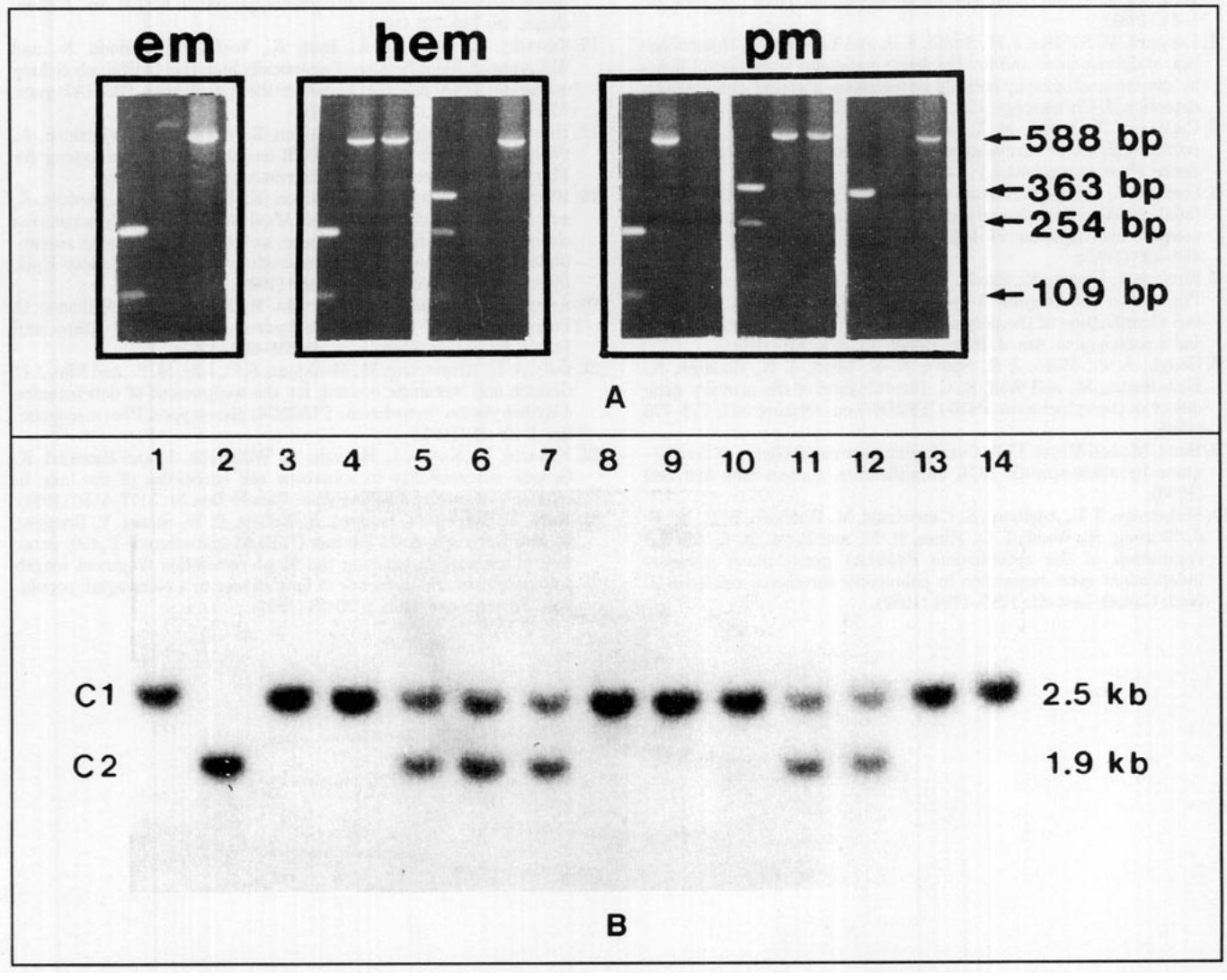

FIGURE 1. Examples of the electrophoresis results from the *CYP2D6* and *CYP1A1* genotyping analyses. (*A*) The six different genotypes from *CYP2D6* PCR-based analysis. Genotypes are resolved by combining the information from the three parallel lanes; EM, homozygous extensive metabolizer genotype; HEM, heterozygous EM genotypes; PM, PM genotypes. (*B*) Autoradiogram of *CYP1A1 Msp*I restriction fragment length polymorphism analysis showing the genotypes *C2C2* (lane 2), *C1C2* (lanes 5–7, 11, 12) and *C1C1* (lanes 1,3,4,8–10,13,14).

evident that more studies involving larger study populations from various ethnic subgroups are needed to draw any conclusions concerning the possible association between polymorphisms in the cytochrome P450 gene superfamily and lung cancer risk.

We thank Tuula Suitiala for her skilled technical assistance and Anne Hand for the linguistic revision.

REFERENCES

1. Nebert, D. W. Role of genetics and drug metabolism in human cancer risk. Mutat. Res. 247: 267–281 (1991).
2. Idle, J. R. Is environmental carcinogenesis modulated by host polymorphism? Mutat. Res. 247: 259–266 (1991).
3. Nebert, D. W., Nelson, D. R., Coon, M. J., Estabrook, R. W., Fayereisen, R., Fujii-Kuriyama, Y., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. W., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R., and Waxman, D. J. The P450 superfamily: Update on new sequences, gene mapping, and recommended nomenclature. DNA Cell Biol. 10: 1–14 (1991).
4. Lennard, M. S., Silas, J. H., Smith, A. J., and Tucker, G. T. Determination of debrisoquine and its 4-hydroxy metabolite in biological fluids by gas chromatography with flame ionization and nitrogen selective detection. J. Chromatogr. 133: 161–166 (1977).
5. Caporaso, N., Landi, M. T., and Vineis, P. Relevance of metabolic polymorphisms to carcinogenesis: evaluation of epidemiologic evidence. Pharmacogenetics 1: 4–19 (1991).
6. Steiner, E., Iselius, L., Alvan, G., Lindsten, J., and Sjoqvist F. A. A family study of genetic and environmental factors determining polymorphic hydroxylation of debrisoquine. Clin. Pharmacol. Ther. 38: 394–400 (1985).
7. Kimura, S., Umeno, M., Skoda, R. C., Meyer, U. A., and Gonzalez, F. J. The human debrisoquine 4-hydroxylase (CYP2D6) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. Am. J. Hum. Genet. 45: 889–904 (1989).
8. Gough, A. C., Miles, J. S., Spurr, N. K., Moss, J. E., Gaedigk, A., Eichelbaum, M., and Wolf, R. C. Identification of the primary gene defect at the cytochrome P450 CYP2D6 locus. Nature 347: 773–776 (1990).
9. Heim, M., and Meyer, U. A. Genotyping poor metabolizers of debrisoquine by allele-specific PCR amplification. Lancet 336: 529–532 (1990).
10. McLemore, T. L., Adelberg, S., Czerwinski, M., Hubbard, W. C., Yu, S. J., Storeng, R., Wood, T. G., Hines, R. N., and Boyd, M. R. Altered regulation of the cytochrome P450IA1 gene: novel inducer-independent gene expression in pulmonary carcinoma cell lines. J. Natl. Cancer Inst. 81: 1787–1794 (1989).
11. McLemore, T. L., Adelberg, S., Liu, M. C., McMahon, N. A., Yu, S. J., Hubbard, W. C., Cerwinski, M., Wood, T. G., Storeng, R., Lubet, R. A., Eggelston, J. C., Boyd, M. R., and Hines, R. N. Expression of CYP1A1 gene in patients with lung cancer: evidence for cigarette smoke induced gene expression in normal lung tissue and for altered gene regulation in primary pulmonary carcinomas. J. Natl. Cancer Inst. 82: 1333–1339 (1990).
12. Kellermann, G., Shaw, C. R., and Luyten-Kellermann, M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. N. Engl. J. Med. 289: 934–937 (1973).
13. Kouri, R. E, McKinney, C. E., Slomiany, D. J., Snodgrass, D. R., Wray, N. P., and McLemore, T. L. Positive correlation between high aryl hydrocarbon hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. Cancer Res. 42: 5030–5037 (1982).
14. Jaiswal, A. K., Gonzalez, F. J., and Nebert, D. W. Human P_1-450 gene sequence and correlation of mRNA with genetic differences in benzo(a)pyrene metabolism. Nucleic Acids Res. 13: 4503–4520 (1985).
15. Spurr, N. K., Gough, A. C., Stevenson, K., and Wolf R. C. Msp-1 polymorphism detected with a cDNA probe for the P-450I family on chromosome 15. Nucleic Acids Res. 15: 5901 (1987).
16. Petersen, D. D., McKinney, C. E., Ikeya, K., Smith, H. H., Bale, A. E., McBride, O. W., and Nebert, D. W. Human CYP1A1 gene: cosegregation of the enzyme inducibility phenotype and an RFLP. Am. J. Hum. Genet. 48: 720–725 (1991).
17. Kawajiri, K., Nakachi, K., Imai, K., Yoshii, A., Shinoda, N., and Watanabe, J. Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450IA1 gene. FEBS Lett. 263: 131–133 (1990).
18. Hirvonen, A., Husgafvel-Pursiainen, K., Anttila, S., Karjalainen, A., Pelkonen, O., and Vainio, H. PCR-based *CYP2D6* genotyping for Finnish lung cancer patients. Pharmacogenetics, in press.
19. Hirvonen, A., Husgafvel-Pursiainen, K., Karjalainen, A., Anttila, S., and Vainio, H. Point mutational *Msp*I and *Ile-Val* polymorphisms closely linked to the *CYP1A1* gene: lack of association with susceptibility to lung cancer in a Finnish study population. Cancer Epid. Biomarkers Prevention 1: 485–489 (1992).
20. Arvela, P., Kirjarinta, M., Kirjarinta, M., Karki, N., and Pelkonen, O. Polymorphism of debrisoquine hydroxylation among Finns and Lapps. Br. J. Clin. Phamacol. 26: 601–603 (1988).
21. Daly, A. K., Armstrong, M., Monkman, S. C., Idle, M. E., and Idle, J. F. Genetic and metabolic criteria for the assignment of debrisoquine 4-hydroxylation (cytochrome P4502D6) phenotypes. Pharmacogenetics 1: 33–41 (1991).
22. Nakachi, K., Kazue, I., Hayashi, S., Watanabe, J., and Kawajiri, K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. Cancer Res. 51: 5177–5180 (1991).
23. Tefre, T., Ryberg, D., Haugen, A., Nebert, D. W., Skaug, V., Brøgger, A., and Børresen, A.-L. Human CYP1A1 (cytochrome $P_1$450) gene: lack of association between the MspI restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. Pharmacogenetics 1: 20–25 (1991).